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UFT and Its Metabolites Inhibit the Angiogenesis Induced by Murine Renal Cell Carcinoma, as Determined by a Dorsal Air Sac Assay in Mice

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ABSTRACT

UFT, an anticancer agent that is composed of tegafur (FT) and uracil at a molar ratio of 1:4, is widely used in clinical practice in Japan to treat cancer patients requiring a long-term chemotherapy, and it is associated with few side effects, if any. In this study, we have evaluated the inhibitory effect of UFT against RENCA cell-induced angiogenesis by a dorsal air sac assay. Marked angiogenesis is induced by implantation of a chamber containing RENCA cells into mice. In this model, UFT showed a strong angiogenesis-inhibitory effect, whereas 5-fluorouracil (5-FU) and doxifluridine were less effective. Additional experiments revealed FT to be effective component of UFT; uracil remained ineffective in the inhibition of angiogenesis. Moreover, we have found that γ-hydroxybutyric acid and γ-butyrolactone, the metabolites of FT, possess a potent angiogenesis inhibitory effect that is amplified when the compounds are administered by a continuous infusion. This may reflect a transition in blood concentration of each metabolite resulting from the administration of UFT. Similar results were also obtained with respect to 5-FU. It was suggested that UFT has a stronger angiogenesis-inhibitory effect than did other fluorinated pyrimidines, partly due to its pharmacokinetic properties characterized by maintaining of higher and long-lasting blood levels of 5-FU and partly due the inhibitory effects derived from γ-hydroxybutyric acid and γ-butyrolactone, UFT-specific metabolites.

INTRODUCTION

Several fluorinated pyrimidines are widely used in the treatment of variety of cancers. UFT is one of frequently used drugs belonging to that class. We previously reported that UFT exhibited a significant life-prolonging effect that was associated with the decreased number of tumor vessels in the metastatic foci in a postoperative adjuvant model of RENCA (murine renal cell carcinoma) cell line (1) . On the other hand, other fluorinated pyrimidines, *e.g.*, 5-FU,[2] a cytotoxic metabolite of FT, and 5'-DFUR, a derivative of 5-FU, neither exerted a significant prolongation of the life span nor affected the number of tumor vessels in treated animals. Furthermore, it was also noted that the drugs with a strong cytotoxicity such as CPT-11 and CDDP and a reference antiangiogenic agent, TNP-470, showed temporary antitumor and angiogenesis-inhibitory effects, but these did not result in significant prolongation of the life span. To achieve a life-prolonging effect, in many instances (2, 3, 4) , it is necessary to administer a drug chronically, a method that is often limited by the toxic effects. Therefore, the balance between efficacy and toxicity is thought to be important to attain survival prolongation. As a matter of fact, only UFT among fluorinated pyrimidines shows, due to its unique biodisposition, a significant life-prolonging effect with simultaneous decreasing of the number of tumor vessels in our experimental model.

This finding seems to be of great importance because an inhibition of angiogenesis will prevent tumor mass enlargement and tumor cell spread into the circulation, thus blocking the formation of metastasis (5, 6, 7) , which, in turn, will determine a survival of a cancer-invaded host organism.

The aims of this study were to: (*a*) provide a direct evidence of the angiogenesis inhibitory effect of UFT; (*b*) compare the efficacy of UFT with those of 5-FU and 5'-DFUR; and (*c*) identify active components derived from UFT, as shown in Fig. 1 , that are responsible for that effect. For that purpose, a DAS assay was used with the induction of angiogenesis by the RENCA cells.

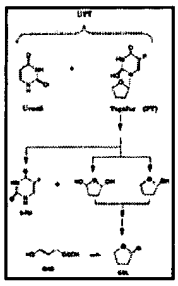

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Fig. 1. Main metabolic pathway of UFT, derived from the experiments in animal species and human patients following p.o. administration.

MATERIALS AND METHODS

Materials and Animals

UFT is a combination of FT and uracil, mixed at the molar ratio of 1:4 (2) . FT was synthesized in our laboratory. Other drugs and reagents were purchased as follows: uracil, GBL, and 5-FU from Wako Pure Chemical Industries (Tokyo, Japan); GHB sodium salt from Sigma Chemical Co. (St. Louis, MO); and 5'-DFUR from Nippon Roche (Tokyo, Japan). Male BALB/cA mice (6 weeks old) were purchased from Charles River Japan (Tokyo, Japan).

UFT, FT, uracil and 5'-DFUR were suspended in 0.5% (w/v) HPMC. 5-FU was dissolved in physiological saline solution for i.v. and i.p. administration and in 7% sodium bicarbonate solution for a continuous infusion study. Both GHB and GBL were dissolved in physiological saline solution. The osmotic pump manufactured by Alzet Inc. (Palo Alto, CA) was used for continuous drug infusion.

Cells

RENCA cell line, a murine renal cell carcinoma, was provided by courtesy of Dr. I. J. Fidler (The University of Texas M. D. Anderson Cancer Center, Houston, TX). It was cultured in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% FBS. MBMECs were purchased from Cell Applications, Inc., and cultured in the exclusive medium provided by the manufacturer.

DAS Assay

The DAS assay was used in mice to examine the effect of UFT and other related compounds on the angiogenic response triggered by RENCA cells, according to a method described by Oikawa *et al.* (8) . Briefly, both sides of a Millipore ring were covered with Millipore filters of 0.45-μm pore size, and the resulting Millipore chamber was filled with the suspension of RENCA cells (10^6 cells) in 0.15 ml of PBS. The RENCA cell-containing chamber was implanted into the preformed air sac in the dorsum of anesthetized (50 mg/kg pentobarbital, i.v.) male BALB/cA mouse. The animals were divided into treated groups on day 0, and the corresponding drugs were administered from day 0 to day 4. On day 5, the implanted chambers were removed from s.c. fascia of treated mice, and then black rings of the same inner diameter as the Millipore rings were placed at the sites exposed to a direct contact with the chamber. The angiogenic response was assessed with a dissecting microscope photographs by determining the number of newly formed blood vessels of >3 mm in length within the area marked by the black ring. The extent of angiogenesis was scored as an index of 0, 1, 2, 3, 4, or 5, indicating zero, one, two, three, four, or five or more newly formed blood vessels, respectively. The blood vessels newly formed by an angiogenic factor(s) released from malignant tumor cells were morphologically distinct from the preexisting background vessels by the zigzagging characteristics (Fig. 2), as described previously (9, 10, 11) .

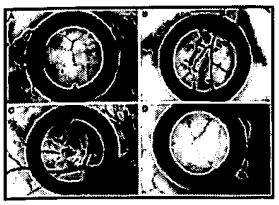

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Fig. 2. The illustration of the angiogenic response induced by RENCA cells in BALB/cA mice and its inhibition by the administration of UFT and 5'-DFUR for 5 consecutive days. The vehicle-treated group was implanted with chambers containing PBS only (*A*), and the control group was implanted with chambers containing RENCA cells (*B*). *Arrowheads*, newly formed vessels, with characteristic zigzagging lines. The number of this type of vessels is reduced by the treatment with 5'-DFUR (0.812 mmol/kg/day; *C*) and almost completely abolished by the treatment with UFT (0.1 mmol/kg/day; *D*).

The following experimental protocols were designed:

Experiment 1.

Experiment 1 involved the evaluation of angiogenesis-inhibitory effects of UFT, 5-FU, and 5'-DFUR. Mice with the implanted chambers containing RENCA cells were divided into six groups ($n = 6$). The positive control group received 0.5% HPMC by p.o. administration. The other three groups of animals were treated p.o. with UFT, at the dosage levels of 0.025, 0.05, and 0.1 mmol/kg/day. These dosages of UFT are expressed as the amount of FT contained in UFT preparation. Two more groups of animals were given either 5-FU i.v. (0.146 mmol/kg/day) or 5'-DFUR p.o. at a dose of 0.812 mmol/kg/day. The negative control group of animals, implanted with the chambers containing only equal volume of PBS without cancer cells, was given a vehicle consisting of 0.5% HPMC. The doses of UFT 0.1 mmol/kg/day and 5'-DFUR 0.812 mmol/kg/day were selected for this experimental design because there had been often used in the treatment protocols of experimental cancers (12 , 13) . That of 5-FU of 0.146 mmol/kg/day is the maximum tolerated dose for five consecutive daily i.v. injections (14) .

Experiment 2.

Experiment 2 evaluated the angiogenesis-inhibitory effects of uracil and FT, the main components of UFT, and of 5-FU, GHB, and GBL, the metabolites of FT. The same procedure as that described for experiment 1 was used, and two control groups (positive controls, $n = 8$; and negative controls, $n = 6$) were used. UFT was p.o. administered at the dosage of 0.1 mmol/kg/day (as the amount of FT), and FT was given p.o. at the dosage of 0.1 and 0.5 mmol/kg/day. Uracil was given p.o., whereas 5-FU, GHB, and GBL were administered i.p. at equimolar dosages corresponding to 0.1 mmol/kg UFT ($n = 6$).

Experiment 3.

The inhibition of angiogenesis by 5-FU, GHB, and GBL, given either as a continuous infusion using osmotic pumps or by repeated i.p. administration ($n = 6$), was comparatively evaluated according to the above method. One group of animals was also treated with concomitant administration of 5-FU and GHB ($n = 6$). The osmotic pump was implanted in the lateral abdominal part of the mouse. The tested compounds were administered at the equimolar doses corresponding to 0.1 mmol/kg of UFT. Control animals were implanted with the osmotic pump containing saline only ($n = 8$).

Experiment 4.

The dose-dependent inhibition of angiogenesis by GHB was also evaluated according to the above methods. GHB was administered by a continuous infusion at the dose of 0.1 mmol/kg/day, corresponding to 0.1 mmol/kg of UFT, and at two additional doses of 0.02 and 0.5 mmol/kg/day, being 5 times decreased

or increased in respect to the middle dose, respectively.

In Vitro Cytotoxicity

The *in vitro* cytotoxicity of the tested compounds against RENCA cells or MBMECs was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after a 72-h incubation (15).

Statistical Analysis

Paired comparison of the angiogenesis indices of the control group and those of the drug groups was conducted by means of two-tailed Wilcoxon's exact test. Differences were considered to be statistically significant if P was ≤ 0.05.

RESULTS

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Inhibition of Angiogenesis by Fluorinated Pyrimidines.

The implantation of the chambers containing only PBS (negative control) was associated with a minimal angiogenesis. The mean angiogenesis index value accounted for only 0.67, indicating that the experimental manipulation and subsequent healing process did not induce a significant angiogenic response. Occasionally, only a single microvessel was presented in some of control animals (Table 1). On the other hand, RENCA cells separated by a semipermeable filter (positive control) triggered the neovascularization process, which was characterized by the angiogenesis index of 4.17. As shown in Table 1 and Fig. 2, UFT suppressed a RENCA cell-induced angiogenesis in a dose-dependent manner. Particularly, the pronounced effect of UFT was observed at the dose level of 0.1 mmol/kg, which is commonly used for the evaluation of the antitumor activity of UFT. The other two drugs tested, namely, 5-FU and 5'-DFUR, showed a minimal and nonsignificant angiogenesis-inhibitory effect. These results indicate that UFT is the strongest inhibitor of angiogenesis among clinically used fluorinated pyrimidines.

View this table: [in this window] [in a new window]	Table 1 Inhibitory effect of UFT, 5-FU, and 5'-DFUR on the angiogenic response induced by RENCA cells in BALB/c mice[a]

Inhibition of Angiogenesis by the Component of UFT and Its Metabolites.

Because only UFT inhibited the angiogenesis induced by RENCA cells, it was essential to identify a component involved in this activity. As shown in Fig. 1, UFT is a mixture of FT and uracil at a molar ratio of 1:4 (16). Under *in vivo* conditions, FT is oxidized and then 5-FU and GHB are released; the latter is in equilibrium with GBL as the result of chemical interconversion (17, 18). The dose-dependent inhibitory effect was observed after administration of FT, the main active component of UFT. The other component, uracil, was completely inactive (Fig. 3). Among metabolic species of UFT, significant inhibition of angiogenesis was observed only after administration of GHB and GBL; however, no significant inhibitory tendency was observed in the case of 5-FU (Fig. 3).

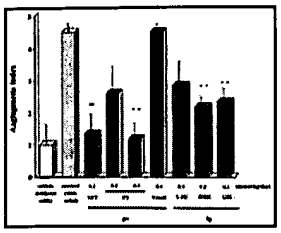

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Fig. 3. Inhibitory effect of UFT, its main components (uracil and FT), and its metabolites (5-FU, GHB, and GBL), on the angiogenic response induced by RENCA cells. The tested compounds were administered daily for 5 days after implantation of a chamber containing RENCA cells at the following doses and routes: UFT (0.1 mmol/kg) and FT (0.1, 0.5 mmol/kg), p.o.; and 5-FU (0.1 mmol/kg), GHB (0.1 mmol/kg), and GBL (0.1 mmol/kg), i.p. Control ($n = 8$) and drug-treated mice ($n = 6$) were implanted with a chamber containing RENCA cells (1×10^6 cells), the vehicle-treated group ($n = 6$) was implanted with a chamber containing only PBS. *Columns*, means; *bars*, SE. **, $P < 0.01$ *versus* controls by two-tailed Wilcoxon exact test.

Angiogenesis-inhibitory Effects of the Metabolites of UFT Given as a Continuous Infusion.

The above-mentioned metabolism of FT takes place in the liver and is mediated by the cytochrome P-450; therefore, the indicated metabolites, namely, 5-FU, GHB, and GBL, appear in the blood in patterns similar to those of compounds that are administered in the form of a sustained-release formulation (17, 18, 19) . In addition, uracil, contained in UFT, prevents further enzymatic degradation of the released 5-FU (16) . To mimic the sustained release pharmacokinetic profile of 5-FU, GHB, and GBL, the main metabolites of UFT, we infused the mentioned compounds by a micro-osmotic pump and compared their inhibitory effects with those observed after i.p. administration. The results shown in Fig. 4 indicate that the drug delivery in the form of a continuous infusion was associated with the stronger inhibition of the angiogenesis by any of the administered compounds.

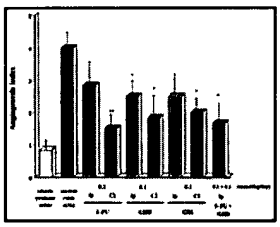

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Fig. 4. Inhibitory effect of 5-FU, GHB, and GBL, the main metabolites of UFT, administered i.p. or by a continuous infusion on the angiogenic response induced by RENCA cells. 5-FU (0.1 mmol/kg), GHB (0.1 mmol/kg), and GBL (0.1 mmol/kg) were administered i.p. once a day or continuously infused by an osmotic pump for 5 days ($n = 6$). The GHB plus 5-FU group ($n = 6$) was coadministered i.p. with GHB and 5-FU once a day for 5 days. Mice were implanted with a chamber containing RENCA cells (1×10^6 cells), and the vehicle-treated group ($n = 6$) was implanted with a chamber containing only PBS. Control animals were implanted with the osmotic pump containing saline only ($n = 8$). *Columns*, means; *bars*, SE. *, $P < 0.05$; **, $P < 0.01$ *versus* controls by two-tailed Wilcoxon exact test.

Angiogenesis-inhibitory Effect of GHB Administered by Continuous Infusion.

GHB inhibited the angiogenesis induced by RENCA cells in a dose-dependent manner (Fig. 5) . When GHB was administered at the maximum dose of 0.5 mmol/kg/day, the angiogenic activity in treated animals returned to the control levels.

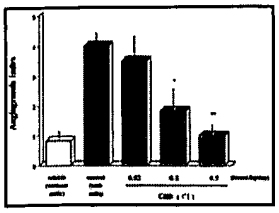

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Fig. 5. Inhibitory effect of GHB on the angiogenic response induced by RENCA cells. GHB was administered as a continuous infusion by an osmotic pump for 5 days at 0.02, 0.1, and 0.5 mmol/kg/day ($n = 6$). Mice were implanted with a chamber containing RENCA cells (1×10^6 cells). The vehicle-treated group ($n = 6$) was implanted with a chamber containing PBS only. Control animals were implanted with the osmotic pump containing saline only ($n = 8$). *Columns*, means; *bars*, SE. *, $P < 0.05$; **, $P < 0.01$ *versus* controls by two-tailed Wilcoxon exact test.

Cytotoxicity Study of 5-FU, GHB, and GBL.

The cytotoxic effects of 5-FU, GHB, and GBL against RENCA cells and MBMECs were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and the results are shown in Fig. 6. The calculated IC_{50}s of 5-FU were determined to be 0.83 and 49 μM for RENCA and MBMEC cells, respectively. On the other hand, both GHB and GBL appeared to be not cytotoxic against both cell lines, even at a concentration of 10 mM.

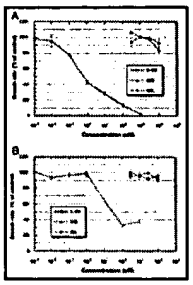

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Fig. 6. Effect of 5-FU, GHB, and GBL on the growth of RENCA cells (*A*) and MBMECs (*B*) *in vitro*. The cells were treated with various concentrations of these compounds for 72 h. The IC_{50}s of 5-FU against RENCA cells and MBMECs were 0.82 and 49 μM, respectively, whereas those of GHB and GBL were not determined because of the lack of obvious cytotoxic effects, even at concentrations exceeding 10 mM.

DISCUSSION

We have previously reported a significant life-prolonging effect of UFT in animals with a metastatic form of RENCA cancer (postoperative adjuvant model). This effect was associated with the decrease of the number of microvessels in the metastatic nodules formed in the lung (1). Kurebayashi *et al.* (20) also reported that UFT significantly inhibited micrometastasis using a postoperative adjuvant model of MKL-4 cells, derived from MCF-7 cell line by the transfection with a *fgf-4* gene, which encodes a potent angiogenic factor. These results suggested that UFT may affect the angiogenesis in the growing tumors and, consequently, prompted us to examine directly whether UFT can inhibit tumor cell-induced angiogenesis.

Among various widely accepted methods used to evaluate the inhibition of angiogenesis (21, 22, 23), the

DAS assay seemed to be the most suitable one in this study. This method has an advantage because the direct observation of angiogenesis induced by tumor cells is possible and is applicable to the compounds, which show an activity after being metabolized. Two compounds under recent evaluation, namely, UFT and 5'-DFUR, are activated *in vivo* to more potent and pharmacologically active metabolites (17 , 24 , 25) . Therefore, it is thought to be more beneficial than the chick embryo chorioallantoic membrane assay or gelatin sponge method (21, 22, 23) .

The results of the evaluation of angiogenesis-inhibitory effects of the fluorinated pyrimidines by DAS assay have shown that UFT is the only compound among them that exerts a strong inhibition of the angiogenesis induced by cancer cells. Significant suppression of angiogenesis was also observed after treatment with FT, a component of UFT preparation (16) . Uracil, the other component of UFT, was without any effect. This led us to the assumption that the angiogenesis-inhibitory effect of UFT may originate from 5-FU, GHB, and GBL, the metabolites of FT (17 , 18) . Surprisingly, it appeared to be true that predominant metabolites of UFT exerted the inhibitory effect, especially those lacking cytotoxicity. The angiogenesis-inhibitory effect of UFT was reconstituted *in vivo* after concomitant i.p. administration of 5-FU and GHB, where at least an additive effect was seen, suggesting that both metabolites of UFT contribute to the inhibition of the neovascularization process (Fig. 4) . Furthermore, we have found that the method of drug delivery essentially contributes to the inhibition of the angiogenesis. The inhibitory effects of GHB, GBL, and 5-FU were more pronounced when they were administered as a continuous infusion, providing a suitable pharmacokinetic profile that is observed after UFT administration. These results suggest that a stronger inhibition of angiogenesis by UFT is attainable by the sustained *in vivo* distribution of GHB/GBL. It is reported in the clinical study that the half-life of GHB is relatively short after a single administration (26 , 27) . However, after administration of UFT, the concentrations of GHB as well as that of 5-FU in the blood are maintained for a relatively long time due to a gradual generation from FT by cytochrome P-450 present in the liver (17, 18, 19) . As shown in this study, such a property is beneficial to the attainment of angiogenesis inhibition.

In the DAS assay, RENCA cells are closed in the filter chamber of 0.45-μm pore size, and the angiogenesis is thought to be induced by angiogenic factors that are produced by RENCA cells. The process of angiogenesis can be roughly separated into the following three steps: growth, migration/invasion, and tube formation by vascular endothelial cells (5, 6, 7) . Because GHB has no cytotoxicity against both RENCA and vascular endothelial cells, it is thought to inhibit the processes other than cell growth. Vasil'eva *et al.* (28) have reported about the effect of GHB on microvessels density as an early phenomenon seen in the celloidin-induced inflammation model in rats. There was no direct evidence of the inhibition of microvasculature formation; rather, the results indicated the presence of unspecified pharmacological effects on the blood supply to the inflammation site. In this study, we have provided the evidence that GHB, a noncytotoxic agent, effectively inhibits tumor-induced neovascularization.

On the other hand, a strong angiogenesis-inhibitory effect of 5-FU was observed after administration of a relatively low dose by a continuous infusion, and the i.p. injections were virtually ineffective. Because the *in vitro* cytotoxic effect of 5-FU on MBMECs is weak (Fig. 6) and only a weak angiogenesis-inhibitory effect was attainable by i.v. bolus administration (Table 1) , one may consider that 5-FU also inhibits angiogenesis by the mechanism different from that of cell growth inhibition. Both UFT and 5'-DFUR are

metabolized to 5-FU, however, after administration of 5'-DFUR the serum levels of 5-FU only temporarily become high (24 , 25 , 29) . On the other hand, the serum levels of 5-FU following UFT administration are sustained for a relatively longer time than those derived from of 5'-DFUR, and the peak levels are not high enough to induce a severe toxic effects (24 , 25 , 29) . Pharmacokinetic profile of 5-FU after administration of UFT is more similar to that of low dose 5-FU given by a continuous infusion. This pharmacokinetic pattern of 5-FU is assumed to play an important role in the inhibition of angiogenesis. We have previously reported that an appropriate dosage of FT has a selective effect on immunocompetent cells and enhances cellular immunity or immune response to the tumor (30 , 31) . The progressive growth of tumors depending on angiogenesis is often associated with an inflammatory process that can be regulated by several chemokines and/or cytokines involved in the tumor angiogenesis (32, 33, 34) . These properties of FT may contribute to the angiogenesis-inhibitory effect of UFT by keeping the antiangiogenic balance in the host.

Angiogenesis is closely associated with tumor mass expansion, and it enhances the tumor's metastasizing ability (5, 6, 7) . The studies of angiogenesis-inhibitory effects of antitumor agents are considered to have significant implications in terms of life prolongation and the improvement of quality of life of patients suffering of cancer. Recently used antitumor agents have a serious limitation in clinical usage due to their strong cytotoxicity and short-lasting therapeutic effects that usually not lead to life-prolonging effects, owing to their severe toxicity (2, 3, 4) . The results of this study have indicated that UFT inhibits tumor-induced angiogenesis and suggested that this effect may contribute to the life prolonging or to antimetastatic effect of UFT in postoperative adjuvant therapy. Also, newly discovered properties of the metabolites of UFT may serve as leading structures for the development of noncytotoxic agents inhibiting the angiogenesis induced by a growing tumor mass. We are now studying in detail the mechanism of angiogenesis inhibition and the population of cancers responding to UFT.

FOOTNOTES

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[2] The abbreviations used are: 5-FU, 5-fluorouracil; FT, tegafur; 5'-DFUR, doxifluridine; DAS, dorsal air sac; GBL, γ-butyrolactone; GHB, γ-hydroxybutyric acid; HPMC, hydroxypropylmethylcellulose; MBMEC, mouse brain endothelial cell. ◘

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1. Shibata J., Murakami K., Abe M., Hashimoto A., Utsugi T., Fukushima M., Yamada Y. Life prolonging effect of antitumor agents on postoperative adjuvant therapy in the lung spontaneous metastasis model in mice. Anticancer Res., *18:* 1203-1210, 1998.[Medline]
2. Einhorn L. H., Loeher P. J., Williams S. D., Meyers S., Gabrys T., Nttan S. R., Woodburn R., Drasga R., Songer J., Fisher W., Stephans D., Hui S. Random prospective study of vindesine *versus* vindesine plus high-dose cisplatin versus vindesine plus cisplatin plus mitomycin C in advanced non-small-lung cancer. J. Clin. Oncol., *4:* 1037-1043, 1986.[Abstract]
3. Gandara D. R., Crowly J., Livingston R. B., Perez E. A., Taylor C. W., Weiss G., Neefe J. R., Hutcins L. F., Roach R. W., Grunberg S. M., Braun T. J., Natale R. B., Balcerzak S. P. Evaluation of cisplatin intensity in metastatic non- small-cell lung cancer: a Phase III study of the Southwest Oncology group. J. Clin. Oncol., *11:* 873-878, 1993.[Abstract]
4. Francis P. A., Rigas J. R., Kris M. G., Pisters K. M. W., Orazem J. P., Woolley K. J., Heelan R. T. Phase II trial of docetaxel in patients with stage III and IV non-small cell lung cancer. J. Clin. Oncol., *12:* 1232-1237, 1994.[Abstract]
5. Ausprunk D. H., Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc. Res., *14:* 53-65, 1977.[Medline]
6. Weinstat-Saslow D., Steeg P. S. Angiogenesis and colonization in the tumor metastatic process: basic and applied advances. FASEB. J., *8:* 401-407, 1994.[Abstract]
7. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nature (Lond.) Med., *1:* 27-31, 1995.
8. Oikawa T., Sasaki M., Inose M., Shimamura M., Kuboki H., Hirano S., Kumagai H., Ishizuka M., Takeuchi T. Effect of Cytogenin, a novel microbial product, on embryonic and tumor cell-induced angiogenic responses *in vivo*. Anticancer Res., *17:* 1881-1886, 1997.[Medline]
9. Sidky Y. A., Borden E. C. Inhibition of angiogenesis by interferons: effects on tumor- and lymphocyte-induced vascular responses. Cancer Res., *47:* 5155-5161, 1987.[Abstract]
10. Downs E. C., Robertson N. E., Riss T. L., Plunkett M. L. Calcium alginate beads as a slow-release system for delivering angiogenic molecules *in vivo* and *in vitro*. J. Cell. Physiol., *152:* 422-429, 1992.[Medline]
11. Majewski S., Szmurlo A., Marczak M., Jablonska S., Bollag W. Synergistic effect of retinoids and interferon α on tumor-induced angiogenesis: anti-angiogenic effect on HPV-harboring tumor cell lines. Int. J. Cancer, *57:* 81-85, 1994.[Medline]
12. Fujita F., Fujita M., Shomozuma K., Hirai T., Taguchi T. Analyses of factors influencing the chemosensitivity of human tumors xenografted into nude mice. Jpn. J. Cancer Chemother., *13:* 3221-3228, 1986.
13. Nio Y., Kimura H., Tsubono M., Tseng C-C., Kawabata K., Masai Y., Hayashi H., Araya S., Meyer C., Fukumoto M. A comparative study of the antitumor activities of 5'-deoxy-5-fluorouridine and its prodrug trimethoxy bezoyl-5'-deoxy-5-fluorouridine (Ro09–1390) on human digestive organ cancer xenograft lines transplanted into nude mice. Anti-Cancer Drugs, *3:* 387-393, 1992.[Medline]
14. Inaba M., Kobayashi T., Tashiro T., Sakurai Y. Pharmacokinetic approach to rational therapeutic doses for human tumor-bearing nude mice. Cancer (Phila.), *79:* 509-516, 1988.
15. Mosmmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, *65:* 55-63, 1983.[Medline]
16. Fujii S., Kitano S., Ikenaka K., Shirasaka T. Studies on coadministration of uracil or cytosine on antitumor activity of FT-207 or 5-FU derivatives. Jpn. J. Cancer Chemother., *6:* 377-384, 1979.
17. Wu A. T., Au J. L., Sadee W. Hydroxylated metabolites of *R,S*-1-(tetrahydro-2-furanyl)-5-fluorouracil (Ftorafur) in rats and rabbits. Cancer Res., *38:* 210-214, 1978.[Abstract]
18. Au J. L., Sadee W. Activation of Ftorafur [*R,S*-1-(tetrahydro-2-furanyl)-5-fluorouracil] to

5-fluorouracil and γ-butyrolactone. Cancer Res., *40:* 2814-2819, 1980.[Medline]
19. Marunaka T., Minami Y., Umeno Y., Yasuda A., Sato T., Fujii S. Identification of metabolites of 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT-207) formed *in vitro* by rat liver. Chem. Pharm. Bull., *28:* 1795-1803, 1980.[Medline]
20. Kurebayashi J., Nukatsuka M., Fujioka A., Saito H., Takeda S., Unemi N., Fukumori H., Kurosumi M., Sonoo H., Dickson R. B. Postsurgical oral administration of uracil and tegafur inhibits progression on micrometastasis of human breast cancer cell in nude mice. Clin. Cancer Res., *3:* 653-659, 1997.[Abstract]
21. Nauyen M., Shing Y., Folkman J. Quantification of angiogenesis and anti-angiogenesis in the chick embryo chorioallantoic membrane. Microvasc. Res., *47:* 31-40, 1994.[Medline]
22. Thompson J. A., Anderson K. D., DiPietro J. M., Zwiebel J. A., Zametta M., Anderson W. F., Maciag T. Site-directed neovessel formation *in vivo*. Science (Washington DC), *241:* 1349-1352, 1988.[Medline]
23. Jain R. K., Schlenger K., Yuan F. Quantification angiogenesis assays: progress and problems. Nature (Lond.), *3:* 1203-1208, 1997.
24. Fujita H., Ogawa K. The tissue distribution and activation of 1-hexylcarbamoyl-5-fluorouracil (HCFU). Rinsyo Yakuri, *12:* 233-243, 1981.
25. Kimura K., Suga S. 5-FU and its derivatives. Jpn. J. Cancer Chemother., *9:* 1321-1326, 1982.
26. Ferrara S. D., Zotti S., Tedeschi L., Frison G., Castagna F., Gallimbert L., Gessa G. L., Palatini P. Pharmacokinetics of γ-hydroxybutyric acid in alcohol-dependent patients after single and repeated oral doses. Br. J. Clin. Pharmacol., *34:* 231-235, 1992.[Medline]
27. Addolorato G., Cibin M., Caprista E., Beghe F., Gessa G., Stefanni G. F., Gasbarrini G. Maintaining abstinence from alcohol with γ-hydroxybutylic acid. Lancet, *351:* 38 1998.[Medline]
28. Vasil'eva L. S., Kuz'menko V. V., Malyshev V. V. Modification of the time course of aseptic inflammation by sodium hydroxybutyrate. Biull. Eksp. Biol. Med., *112:* 1553-1555, 1991.
29. Fujita H., Ogawa K., Nakagawa H., Kawaguchi K., Nakagawa Y., Doi Y. Pharmacokinetics of 5'-deoxy-5-fluorouridine (5'-DFUR). J. Jpn. Soc. Cancer Ther., *18:* 916-926, 1983.
30. Yamada Y., Kawamura T., Gotohda E., Akiyama J., Hosokawa M., Kodama T., Kobayashi H. Effect of normal allogeneic lymphoid cell transfer in combination with chemotherapy on a transplantable tumor in rats. Cancer Res., *40:* 954-958, 1980.[Abstract]
31. Yamada Y., Miyauchi S., Unemi N. Differential effects of 5-fluorouracil analogs on humoral and cell-mediated responses in mice: augmentation of delayed hypersensitivity response against picryl chloride by 1,3-bis(tetrahydro-2-furanyl)-5-fluoro-2,4-pyrimidinedione (FD-1), a new anti-tumor drug. J. Clin. Lab. Immunol., *4:* 153-157, 1980.[Medline]
32. Griffioen A. W., Damen C. A., Blijham G. H., Groenewegen G. Tumor angiogenesis is accompanied by a decreased inflammatory response of tumor-associated endothelium. Blood, *88:* 667-673, 1996.[Abstract/Free Full Text]
33. Watanabe M., McCormick K. L., Volker K., Ortaldo J. R., Wigginton J. M., Brunda M. J., Wiltrout R. H., Fogler W. E. Regulation of local host-mediated anti-tumor mechanisms by cytokines: direct and indirect effects on leukocyte recruitment and angiogenesis. Am. J. Pathol., *150:* 1869-1880, 1997.[Abstract]
34. Gupta S. K., Lysko P. G., Pillarisetti K., Ohlstein E., Stadel J. M. Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. J. Biol. Chem., *273:* 4282-4287, 1998.[Abstract/Free Full Text]

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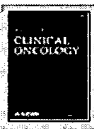
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